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4-Toluenesulfonylureido derivatives of amines, amino acids and dipeptides: a novel class of potential antitumor agents

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Abstract

The screening of a series of arylsulfonylureido derivatives of amines (such as histamine, or dopamine), aliphatic/aromatic amino acids (such as Gly, β-Ala, Val, Lys, Arg, Phe, Tyr, DOPA, etc.) and dipeptides (such as GlyGly, β-AlaHis) led to the identification of three derivatives that possess tumor growth inhibitory properties against several leukemia, non-small cell lung, ovarian, melanoma, colon, CNS, renal, and breast cancer cell lines in vitro. The new derivatives were prepared by reaction of 4-toluenesulfonyl isocyanate with (protected) amines, amino acids or dipeptides. The mechanism of antitumor action with these new derivatives is not known at the moment but it may imply uncoupling of mitochondria, as for the structurally related diarylsulfonylurea sulofenur, an investigational anticancer agent.

Keywords: 4-Toluenesulfonylureas; Amino acid; Dipeptide; Histamine; Dopamine; Antitumor action

1. Introduction

A common feature of several types of antitumor agents reported in recent years is the presence of primary/secondary sulfonamide moieties in their molecules (Howbert et al., 1990; Mohamadi et al., 1992; Chern et al., 1997; Toth et al., 1997; Medina et al., 1998, 1999). Thus, some diarylsulfonyl-ureas/hydroxyguanidines (Howbert et al., 1990; Mohamadi et al., 1992) and diarylsulfonimideamides (Toth et al., 1997) of types **1**–**3** have been reported by researchers from Eli Lilly and by Chern et al. (1997) to possess potent cytotoxic properties, whereas the *N*-substituted polyhalogeno-benzenesulfonamides (Medina et al., 1998, 1999) of type **4** were shown to strongly inhibit the growth of several tumors, among which are the multidrug resistant MCF-7/ADR cancer cells, in vitro and in vivo. Other antitumor sulfonamides that have been investigated include CQS, 5-chloroquinoxaline-2-sulfanilamide (**5**) (Branda et al., 1988; Rigas et al., 1995), E7010 (**6**) (Yoshino et al., 1992) and E7070 (**7**) (Owa et al., 1999), the last compound being in phase I clinical studies in Europe. Our group recently reported powerful tumor growth inhibition with sulfonamides incorporating alkyldithiocarbamyl moieties of types **8** and **9** (Scozzafava and Supuran, 2000a; Supuran et al., 2000). The mechanisms of antitumor action of many of these compounds are unclear (Rush et al., 1992), but E7010 (**6**) and the perfluoroarylsulfonyl derivatives (**4**) were shown to act as tubulin polymerization inhibitors, binding at the colchicine site, and covalently modifying Cys 239 of this protein, respectively (Medina et al., 1999; Flygare et al., 1998; Yoshino et al., 1992; Sirotnak et al., 2000), whereas the molecular targets of sulfonamides **8**, **9** and E7070 may be some carbonic anhydrase (CA) isozymes predominantly present in tumor cells (Supuran and Scozzafava, 2000).

It is generally assumed that the strong cytotoxicity and, as a consequence, the antitumor properties of the diarylsulfonylurea derivatives **1**–**3** is due to the uncoupling of mitochondria (Houghton and Houghton, 1996; Sosinski et al., 1994), but other mechanisms, such as, for instance, inhibition of the mitochondrial isozyme V of carbonic anhydrase (CA V) have also been hypothesized, since hydrolysis of the cytotoxic agent, leading to the formation of unsubstituted sulfonamides as the principal products,

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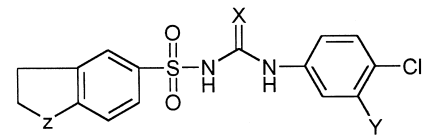
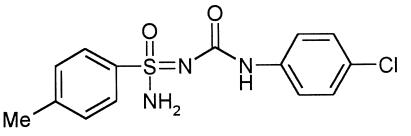


1a: X = O, Y = H, Z = CH_2 - sulofenur
1b: X = O, Y = Cl, Z = O - LY295501
1c: X = S, Y = H, Z = CH_2



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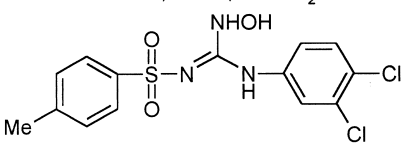

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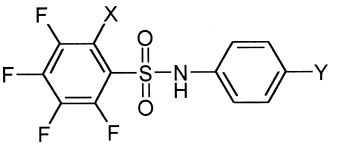
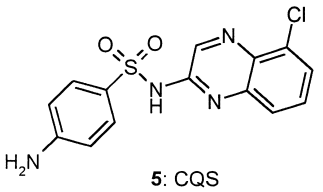
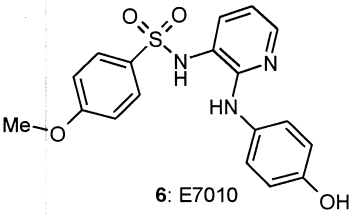


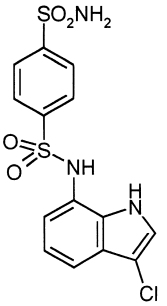
4: X = H, F, Br
Y = OMe; NH_2; NMe_2



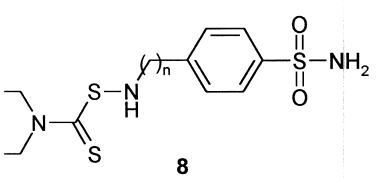
5: CQS



6: E7010



7: E7070



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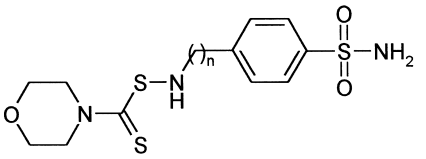


9

n = 0
n = 1
n = 2

GI_{50} = 10 - 65 nM
(different tumor cell lines)

has been reported both in vivo and in vitro (Ehlhardt, 1991). It is well known that aromatic/heterocyclic sulfonamides (formed after such a hydrolytic process) act as very potent inhibitors of CAs (Supuran and Scozzafava, 2000; Scozzafava et al., 1999a,b), and these enzymes are involved in a multitude of crucial physiologic processes (Supuran and Scozzafava, 2000).

The interesting antitumor properties of derivatives **1**–**3** possessing diarylsulfonyl urea moieties (or their structurally related variants) prompted us to investigate in some detail this relatively unexplored class of potential antitumor agents. Here we report the synthesis of some arylsulfonylureido derivatives of physiologically important autacoids such as histamine or dopamine, of several aliphatic/aromatic amino acids or dipeptides, as well as their in vitro tumor cell growth inhibitory properties against several leukemia, non-small cell lung, ovarian, melanoma, colon, CNS, renal, and breast cancer cell lines. The drug design was carried out by considering derivatives of type **1** as lead molecules, in which the arylsulfonylureido part has been maintained constant (i.e., only tosylureido derivatives have been obtained), whereas the groups substituting the other urea nitrogen atom were varied, including aliphatic/aromatic amino acid, dipeptide and heterocyclic amine derivatives among the investigated compounds.

2. Materials and methods

2.1. Chemistry

Melting points were determined with a heating plate microscope and are not corrected. ^{1}H-NMR spectra were recorded with a Varian 300CXP with DMSO-d_6 as solvent. ^{13}C-NMR spectra were registered at 75 MHz with the same apparatus and solvent. Chemical shifts are expressed as δ values relative to Me_4Si as standard. Attributions were done by means of chemical shifts, peak integration, COSY (^{1}H–^{1}H), HETCOR (^{1}H–^{13}C), attached proton test (APT), model spectra and selective deuteration. Elemental analyses were peformed by combustion for C, H and N with an automated Carlo Erba analyzer, and were ±0.4% of the theoretical values. Preparative HPLC was carried out on C_{18} reversed-phase Bondapack or Dynamax-60A (25× 250 mm) columns, with a mobile phase of 90% acetonitrile/7% methanol/3% water (30 ml/min) and fractions of 5 ml collected continuously.

Compounds used in synthesis (histamine, dopamine, natural and non-natural amino acids, trityl/tritylsulfenyl chloride, 9-fluorenylmethoxycarbonil (fmoc) protected amino acids, fmoc-chloroformate, piperidine, etc.) were commercially available compounds (from Sigma–Aldrich or Acros). The 4-methylphenylsulfonylureido-amine/amino acid/dipeptide derivatives were prepared as described previously (Scozzafava and Supuran, 1999, 2000b) by the reaction of 4-tosyl isocyanate (Acros) with amines/amino acids/dipeptides (from Sigma or Aldrich) eventually protected at other functional groups present in their molecules. Acetonitrile, acetone, dioxane (Merck) and other solvents used in the syntheses were doubly distilled and kept on molecular sieves in order to maintain them in an anhydrous conditions.

2.1.1. Preparation of O-trityl-protected dopamine, Tyr and DOPA

An amount of 5 mM fmoc-dopamine, fmoc-Tyr or fmoc-DOPA and 1.55 g (5 mM) trityl chloride (in the case of dopamine and DOPA a double amount was used, since both phenolic moieties have to be protected) were suspended in 100 ml of anhydrous acetonitrile, and 1.40 ml (10 mM) triethylamine was added dropwise. The mixture was stirred at room temperature for 3–5 h (TLC control), the solvent was then evaporated and the crude products stirred with 100 ml water and ice. The tan precipitates obtained were filtered, dried and used directly in the next step, i.e. deprotection of the amino group. This was performed by treatment with piperidine (Fields and Noble, 1990), where the fmoc moiety is removed without affecting the trityl moiety, which is hydrolyzed under acidic conditions (HCl/dioxane, triflic acid/dioxane, etc.). The obtained trityl derivatives could be used directly in the tosylation step as described below, whereas the trityl moiety was removed at the end by acidic hydrolysis, as described previously for the tritylsulfenyl derivatives of histamine (Briganti et al., 1999; Scozzafava and Supuran, 1999, 2000b).

2.1.2. General procedure for the preparation of 4-methylphenylsulfonylureido amines/amino acids/dipeptides (ts-AMN, ts-AA and ts-AA1-AA2) ***10a–k, 10g′, 10h′, 10l′*** *and* ***10m′***

An amount of 10 mM amine/amino acid/dipeptide (eventually protected at the other functional groups that might react with tosyl isocyanate) was suspended/dissolved in 50 ml anhydrous acetone or acetonitrile, and a stoichiometric amount of 4-tosyl isocyanate was added in one portion with energetic stirring and cooling of the reaction mixture. The mixture was then stirred for 1–2 h at 4°C, the solvent was evaporated in vacuo and the product purified either by recrystallization from water–ethanol (1:1, v/v), or by preparative HPLC (in the case of ts-GlyGly, ts-Val, ts-Trp and ts-Phe, where the arylsulfonylureido-amino acid/dipeptide contained variable amounts of unreacted amino acid and 4-toluenesulfonamide). Conditions: C_{18} reversed-phase Bondapack or Dynamax-60A (25×250 mm) column; 90% acetonitrile/8% ethanol/2% water; 30 ml/min.

4-Toluenesulfonylureido-glycine, ts-Gly (**10a**) (as white crystals): ^{1}H-NMR: 2.63 (s, 3H, C$\underline{H}_3C_6H_4$), 3.67 (s, 2H, C$\underline{H}_2$), 7.65 (d, $^{3}J_{HH}$ = 8.1, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 7.99 (d, $^{3}J_{HH}$ = 8.1, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 26.1 ($\underline{C}H_3C_6H_4$), 40.8 ($\underline{C}H_2$ of Gly), 130.9 ($\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.4 (NH$\underline{C}$ONH), 135.0 ($\underline{C}_{ortho}$ of $CH_3C_6H_4$), 145.0 ($\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.6 ($\underline{C}_{para}$ of $CH_3C_6H_4$), 177.3 ($\underline{C}O_2H$ of Gly). Analysis, found: C, 44.35; H, 4.13; N, 10.06%; $C_{10}H_{12}N_2O_5S$ (M=272.282) requires: C, 44.11; H, 4.44; N, 10.29%.

4-Toluenesulfonylureido-β-alanine, ts-β-Ala (**10b**) (as white crystals): ^{1}H-NMR: 2.60 (3H, C$\underline{H}_3C_6H_4$), 2.73 (t, $^{3}J_{HH}$ = 6.6, 1H, $CH_2C\underline{H}_2CO_2H$ of β-Ala), 3.27 (t, $^{3}J_{HH}$ = 6.7, 1H, $CH_2C\underline{H}_2CO_2H$ of β-Ala), 3.43 (t, $^{3}J_{HH}$ = 6.3, 2H, $C\underline{H}_2CH_2CO_2H$ of β-Ala), 7.62 (d, $^{3}J_{HH}$ = 8.2, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 8.00 (d, $^{3}J_{HH}$ = 8.2, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 26.1 ($\underline{C}H_3C_6H_4$), 37.5 (NH$\underline{C}H_2CH_2$ of β-Ala), 40.9 ($CH_2\underline{C}H_2CO_2H$ of β-Ala), 130.8 ($\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.3 (NH$\underline{C}$ONH), 135.0 ($\underline{C}_{ortho}$ of $CH_3C_6H_4$), 144.8 ($\underline{C}_{para}$ of $CH_3C_6H_4$), 148.7 ($\underline{C}_{ipso}$ of $CH_3C_6H_4$), 180.1 ($\underline{C}O_2H$ of β-Ala). Analysis, found: C, 45.91; H, 4.96; N, 9.50%; $C_{11}H_{14}N_2O_5S$ (M= 286.309) requires: C, 46.15; H, 4.93; N, 9.78%.

4-Toluenesulfonylureido-L-valine, ts-Val (**10c**) (as white crystals): ^{1}H-NMR: 1.11 (d, $^{3}J_{HH}$ = 6.7, 6H, CH(C$\underline{H}_3$)$_2$ of Val), 2.29–2.55 (m, 1H, C$\underline{H}$(CH$_3$)$_2$ of Val), 2.70 (s, 3H, C$\underline{H}_3C_6H_4$), 3.75 (d, $^{3}J_{HH}$ = 4.3, 1H, NHC$\underline{H}$CH of Val), 7.72 (d, $^{3}J_{HH}$ = 8.2, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 8.04 (d, $^{3}J_{HH}$ = 8.2, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 22.3 (CH($\underline{C}H_3$)$_2$ of Val), 23.5 (CH($\underline{C}H_3$)$_2$ of Val), 26.1 ($\underline{C}H_3C_6H_4$), 34.0 ($\underline{C}$H(CH$_3$)$_2$ of Val), 64.4 (NH$\underline{C}$HCH of

Val), 130.8 ($\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.3 (NH$\underline{C}$ONH), 134.9 ($\underline{C}_{ortho}$ of $CH_3C_6H_4$), 145.0 ($\underline{C}_{para}$ of $CH_3C_6H_4$), 148.4 ($\underline{C}_{ipso}$ of $CH_3C_6H_4$), 178.8 ($\underline{C}O_2H$ of Val). Analysis, found: C, 49.72; H, 6.02; N, 8.80%; $C_{13}H_{18}N_2O_5S$ (M = 314.362) requires: C, 49.67; H, 5.77; N, 8.91%.

α-4-Toluenesulfonylureido-L-lysine hydrochloride, ts-Lys·HCl (**10d**) (as white crystals): ^{1}H-NMR: 1.66–2.20 (m, 6H, CH($C\underline{H}_2$)$_3$$CH_2$ of Lys), 2.61 (s, 3H, $C\underline{H}_3C_6H_4$), 3.13 (t, $^3J_{HH}$ = 6.7, 2H, $CH_2C\underline{H}_2NH_2$ of Lys), 3.84 (t, $^3J_{HH}$ = 6.7, 1H, $CH_2C\underline{H}$(NH)CO_2H of Lys), 7.63 (d, $^3J_{HH}$ = 8.1, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 7.98 (d, $^3J_{HH}$ = 8.1, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 26.0 ($\underline{C}H_3C_6H_4$), 26.6 ($H_2NCH_2(\underline{C}H_2)_3$ of Lys), 31.4 ($H_2NCH_2(\underline{C}H_2)_3$ of Lys), 34.8 ($H_2NCH_2(\underline{C}H_2)_3$ of Lys), 43.8 ($H_2N\underline{C}H_2(CH_2)_3$ of Lys), 58.8 ($CH_2\underline{C}H$(NH)CO_2H of Lys), 130.8 ($\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.4 (NH$\underline{C}$ONH), 135.0 ($\underline{C}_{ortho}$ of $CH_3C_6H_4$), 144.9 ($\underline{C}_{para}$ of $CH_3C_6H_4$), 148.5 ($\underline{C}_{ipso}$ of $CH_3C_6H_4$), 177.6 ($\underline{C}O_2H$ of Lys). Analysis (of the free base, obtained by neutralization of the hydrochloride with $NaHCO_3$), found: C, 48.61; H, 6.36; N, 12.08%; $C_{14}H_{21}N_3O_5S$ (M = 343.404) requires: C, 48.97; H, 6.16; N, 12.24%.

α-4-Toluenesulfonylureido-L-arginine hydrochloride, ts-Arg·HCl (**10e**) (as white crystals): ^{1}H-NMR: 1.70–2.00 (m, 2H, $CHCH_2C\underline{H}_2$ of Arg), 2.50–2.58 (m, 2H, $CHC\underline{H}_2CH_2$ of Arg), 2.63 (s, 3H, $C\underline{H}_3C_6H_4$), 3.30–3.45 (m, 2H, $CH_2C\underline{H}_2NH$ of Arg), 3.45–3.60 (m, 1H, $CH_2C\underline{H}$(NH)CO_2H of Arg), 7.64 (d, $^3J_{HH}$ = 8.1, 2H, $\underline{H}_{ortho}$ of $C\underline{H}_3C_6H_4$), 7.98 (d, $^3J_{HH}$ = 8.1, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 26.0 ($\underline{C}H_3C_6H_4$), 29.7 ($CH_2\underline{C}H_2CH_2$ of Arg), 35.4 ($CHCH_2\underline{C}H_2$ of Arg), 45.7 ($CH_2\underline{C}H_2NH$ of Arg), 59.8 ($CH_2\underline{C}H$(NH)CO_2H of Arg), 130.8 ($\underline{C}_{meta}$ of $CH_3C_6H_4$), 131.5 (NH$\underline{C}$ONH), 134.9 ($\underline{C}_{ortho}$ of $CH_3C_6H_4$), 145.0 ($\underline{C}_{para}$ of $CH_3C_6H_4$), 148.4 ($\underline{C}_{ipso}$ of $CH_3C_6H_4$), 161.5 (NH$\underline{C}$(NH)NH_2 of Arg), 183.2 ($\underline{C}O_2H$ of Arg). Analysis (of the free base, obtained by neutralization of the hydrochloride with $NaHCO_3$), found: C, 45.51; H, 5.49; N, 18.66%; $C_{14}H_{21}N_5O_5S$ (M = 371.417) requires: C, 45.27; H, 5.70; N, 18.86%.

4-Toluenesulfonylureido-L-phenylalanine, ts-Phe (**10f**) (as white crystals): ^{1}H-NMR: 2.65 (s, 3H, $C\underline{H}_3C_6H_4$), 3.10–3.55 (m, 2H, $C\underline{H}_2CH$ of Phe), 4.08 (dd, $^3J_{HH}$ = 5.0, $^3J_{HH}$ = 7.8, 1H, $CH_2C\underline{H}$ of Phe), 7.29–7.58 (m, 7H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$ and $\underline{H}_{arom}$ of Phe), 7.95 (d, $^3J_{HH}$ = 8.3, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 26.2 ($\underline{C}H_3C_6H_4$), 41.7 ($\underline{C}H_2CH$ of Phe), 59.3 ($CH_2\underline{C}H$ of Phe), 130.9 ($\underline{C}_{para}$ of Phe), 132.3 ($\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.7 (NH$\underline{C}$ONH), 133.8 ($\underline{C}_{meta}$ of Phe), 134.4 ($\underline{C}_{ortho}$ of Phe), 135.1 ($\underline{C}_{ortho}$ of $CH_3C_6H_4$), 141.5 ($\underline{C}_{ipso}$ of Phe), 145.0 ($\underline{C}_{para}$ of $CH_3C_6H_4$), 148.6 ($\underline{C}_{ipso}$ of $CH_3C_6H_4$), 178.6 ($\underline{C}O_2H$ of Phe). Analysis, found: C, 56.41; H, 4.85; N, 7.60%; $C_{17}H_{18}N_2O_5S$ (M = 349.39) requires: C, 56.34; H, 5.01; N, 7.73%.

4-Toluenesulfonylureido-L-tyrosine, ts-Tyr (**10g**) (as pale tan crystals): ^{1}H-NMR: 2.61 (s, 3H, $C\underline{H}_3C_6H_4$), 3.12–3.59 (m, 2H, $C\underline{H}_2CH$ of Tyr), 4.13 (dd, $^3J_{HH}$ = 5.0, $^3J_{HH}$ = 7.8, 1H, $CH_2C\underline{H}$ of Tyr), 7.21–7.69 (m, 6H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$ and $\underline{H}_{arom}$ of Tyr), 7.98 (d, $^3J_{HH}$ = 8.3, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 26.0 ($\underline{C}H_3C_6H_4$), 41.3 ($\underline{C}H_2CH$ of Tyr), 59.8 ($CH_2\underline{C}H$ of Tyr), 130.9 ($\underline{C}_{para}$ of Tyr), 132.2 ($\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.9 (NH$\underline{C}$ONH), 133.5 ($\underline{C}_{meta}$ of Tyr), 134.8 ($\underline{C}_{ortho}$ of Tyr), 135.5 ($\underline{C}_{ortho}$ of $CH_3C_6H_4$), 145.1 ($\underline{C}_{para}$ of $CH_3C_6H_4$), 146.5 ($\underline{C}_{ipso}$ of Tyr), 148.6 ($\underline{C}_{ipso}$ of $CH_3C_6H_4$), 178.9 ($\underline{C}O_2H$ of Tyr). Analysis, found: C, 53.90; H, 4.62; N, 7.25%; $C_{17}H_{18}N_2O_6S$ (M = 378.41) requires: C, 53.96; H, 4.79; N, 7.40%.

4-Toluenesulfonylureido-L-2,3-dioxyphenylalanine, ts-DOPA (**10h**) (as tan crystals): ^{1}H-NMR: 2.65 (s, 3H, $C\underline{H}_3C_6H_4$), 3.08–3.54 (m, 2H, $C\underline{H}_2CH$ of DOPA), 4.16 (dd, $^3J_{HH}$ = 5.0, $^3J_{HH}$ = 7.8, 1H, $CH_2C\underline{H}$ of DOPA), 7.09–7.66 (m, 5H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$ and $\underline{H}_{arom}$ of DOPA), 7.95 (d, $^3J_{HH}$ = 8.3, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 26.2 ($\underline{C}H_3C_6H_4$), 41.5 ($\underline{C}H_2CH$ of Phe), 60.5 ($CH_2\underline{C}H$ of DOPA), 132.2 ($\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.9 (NH$\underline{C}$ONH), 133.5 ($\underline{C}_{meta}$ of DOPA), 134.3 ($\underline{C}_{para}$ of DOPA), 134.8 ($\underline{C}_{ortho}$ of DOPA), 135.5 ($\underline{C}_{ortho}$ of $CH_3C_6H_4$), 135.8 ($\underline{C}_{meta(OH)}$ of DOPA), 145.0 ($\underline{C}_{para}$ of $CH_3C_6H_4$), 147.9 ($\underline{C}_{ipso}$ of DOPA), 148.6 ($\underline{C}_{ipso}$ of $CH_3C_6H_4$), 180.6 ($\underline{C}O_2H$ of DOPA). Analysis, found: C, 51.64; H, 4.39; N, 7.03%; $C_{17}H_{18}N_2O_7S$ (M = 394.41) requires: C, 51.77; H, 4.60; N, 7.10%.

4-Toluenesulfonylureido-L-tryptophan, ts-Trp (**10i**) (as white crystals): ^{1}H-NMR: 2.62 (s, 3H, $C\underline{H}_3C_6H_4$), 3.44 (dd, $^3J_{HH}$ = 9.0, $^2J_{HH}$ = 14.6, 1H, $C\underline{H}_2CH$ of Trp), 3.65 (dd, $^3J_{HH}$ = 4.1, $^2J_{HH}$ = 15.0, 1H, $C\underline{H}_2CH$ of Trp), 4.14 (dd, $^3J_{HH}$ = 4.3, $^3J_{HH}$ = 8.0, 1H, $CH_2C\underline{H}$ of Trp), 7.22–7.82 (m, 7H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$ and $\underline{H}_{arom}$ of Trp), 7.92 (d, $^3J_{HH}$ = 8.2, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 26.0 ($\underline{C}H_3C_6H_4$), 31.6 ($\underline{C}H_2CH$ of Trp), 58.8 ($CH_2\underline{C}H$ of Trp), 113.9 ($\underline{C}$-2 of Trp), 116.9 ($\underline{C}$-7 of Trp), 123.4 ($\underline{C}$-5 of Trp), 124.3 ($\underline{C}$-6 of Trp), 126.8 ($\underline{C}$-4 of Trp), 129.0 ($\underline{C}$-1 of Trp), 130.9 ($\underline{C}_{meta}$ of $CH_3C_6H_4$), 132.1 ($\underline{C}$-8 of Trp), 132.2 (NH$\underline{C}$ONH), 135.0 ($\underline{C}_{ortho}$ of $CH_3C_6H_4$), 141.2 ($\underline{C}$-3 of Trp), 145.0 ($\underline{C}_{para}$ of $CH_3C_6H_4$), 148.6 ($\underline{C}_{ipso}$ of $CH_3C_6H_4$), 179.0 ($\underline{C}O_2H$ of Trp). Analysis, found: C, 56.93; H, 4.54; N, 10.31%; $C_{19}H_{19}N_3O_5S$ (M = 401.443) requires: C, 56.85; H, 4.77; N, 10.47%.

4-Toluenesulfonylureido-glycylglycine, ts-Gly–Gly (**10j**) (as white crystals): ^{1}H-NMR: 2.49 (s, 4H, 2 $C\underline{H}_2$), 2.73 (s, 3H, $C\underline{H}_3C_6H_4$), 7.75 (d, $^3J_{HH}$ = 8.2, 2H, $\underline{H}_{ortho}$ of $CH_3C_6H_4$), 8.08 (d, $^3J_{HH}$ = 8.2, 2H, $\underline{H}_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 26.0 ($\underline{C}H_3C_6H_4$), 35.7 ($\underline{C}H_2$ of Gly), 130.8 ($\underline{C}_{meta}$ of $CH_3C_6H_4$), 134.9 ($\underline{C}_{ortho}$ of $CH_3C_6H_4$), 145.0 ($\underline{C}_{ipso}$ of $CH_3C_6H_4$), 148.4 ($\underline{C}_{para}$ of $CH_3C_6H_4$). Analysis, found: C, 43.70; H, 4.82; N, 12.54%; $C_{12}H_{15}N_3O_6S$ (M = 329.334) requires: C, 43.76; H, 4.59; N, 12.76%.

4-Toluenesulfonylureido-carnosine, ts-β-Ala–His (ts-carnosine) (**10k**) (as white crystals): ^{1}H-NMR: 2.63 (s, 3H, $C\underline{H}_3C_6H_4$), 2.79–2.88 (m, 2H, $C\underline{H}_2$ of β-Ala), 3.11–3.26 (m, 2H, $C\underline{H}_2$ of β-Ala), 3.34–3.45 (m, 2H, $CHC\underline{H}_2$ of His), 4.57–4.63 (m, 1H, $C\underline{H}CH_2$ of His), 7.32 (s, 1H, $C\underline{H}$-5

of His), 7.56 (d, $^3J_{HH}$ = 8.1, 2H, H$_{ortho}$ of $CH_3C_6H_4$), 7.87 (d, $^3J_{HH}$ = 8.1, 2H, H$_{meta}$ of $CH_3C_6H_4$), 8.35 (s, 1H, CH-2 of His). ^{13}C-NMR: 25.9 ($CH_3C_6H_4$), 33.3 (CH_2 of His), 37.4 ($NHCH_2CH_2$ of β-Ala)), 40.8 (CH_2CH_2CO of β-Ala), 59.6 ($CHCH_2$ of His), 122.2 (C-4 of His), 130.6 (C$_{meta}$ of $CH_3C_6H_4$), 131.8 (NHCONH), 134.2 (C-5 of His), 135.2 (C$_{ortho}$ of $CH_3C_6H_4$), 137.2 (C-2 of His), 145.6 (C$_{para}$ of $CH_3C_6H_4$), 149.1 (C$_{ipso}$ of $CH_3C_6H_4$), 175.6 (CH_2CO of β-Ala), 180.4 (CO_2H of His). Analysis, found: C, 48.36; H, 5.13; N, 16.29%; $C_{17}H_{21}N_5O_6S$ (M = 423.450) requires: C, 48.22; H, 5.00; N, 16.54%.

4-Toluenesulfonylureido-histamine, ts-Hsn (**10l**). This compound has been reported previously (Briganti et al., 1999) but was not tested for its antitumor properties (it was prepared as an activator of the enzyme carbonic anhydrase). The antitumor data are reported in this paper.

4-Toluenesulfonylureido-dopamine, ts-Dpn (**10m**) (as tan crystals): ^{1}H-NMR: 2.61 (s, 3H, $CH_3C_6H_4$), 3.08–3.54 (t, 2H, CH_2CH_2N), 4.54 (t, 2H, CH_2CH_2N), 7.13–7.68 (m, 5H, H$_{ortho}$ of $CH_3C_6H_4$ and H$_{arom}$ of Dpn), 7.97 (d, $^3J_{HH}$ = 8.3, 2H, H$_{meta}$ of $CH_3C_6H_4$). ^{13}C-NMR: 26.1 ($CH_3C_6H_4$), 41.4 (CH_2CH_2N), 53.9 (CH_2CH_2N), 132.1 (C$_{meta}$ of $CH_3C_6H_4$), 133.0 (NHCONH), 133.7 (C$_{meta}$ of Dpn), 134.2 (C$_{ortho}$ of Dpn), 134.6 (C$_{para}$ of Dpn), 135.2 (C$_{ortho}$ of $CH_3C_6H_4$), 135.9 (C$_{meta(OH)}$ of Dpn), 145.0 (C$_{para}$ of $CH_3C_6H_4$), 147.1 (C$_{ipso}$ of Dpn), 148.4 (C$_{ipso}$ of $CH_3C_6H_4$). Analysis, found: C, 55.13; H, 4.90; N, 7.86%; $C_{16}H_{18}N_2O_5S$ (M = 306.40) requires: C, 54.85; H, 5.18; N, 7.99%.

2.2. Inhibition of tumor cell growth

Stock solutions of the test compound (1 mM) were prepared in DMSO, and dilutions up to 10 nM were made with distilled deionized water. The control of the cell growth assay was DMSO. The percentage growth (PG) of the cell lines in the presence of five concentrations (10^{-8}–10^{-4} M) of inhibitor was calculated according to one of the following two expressions (Teicher, 1997):

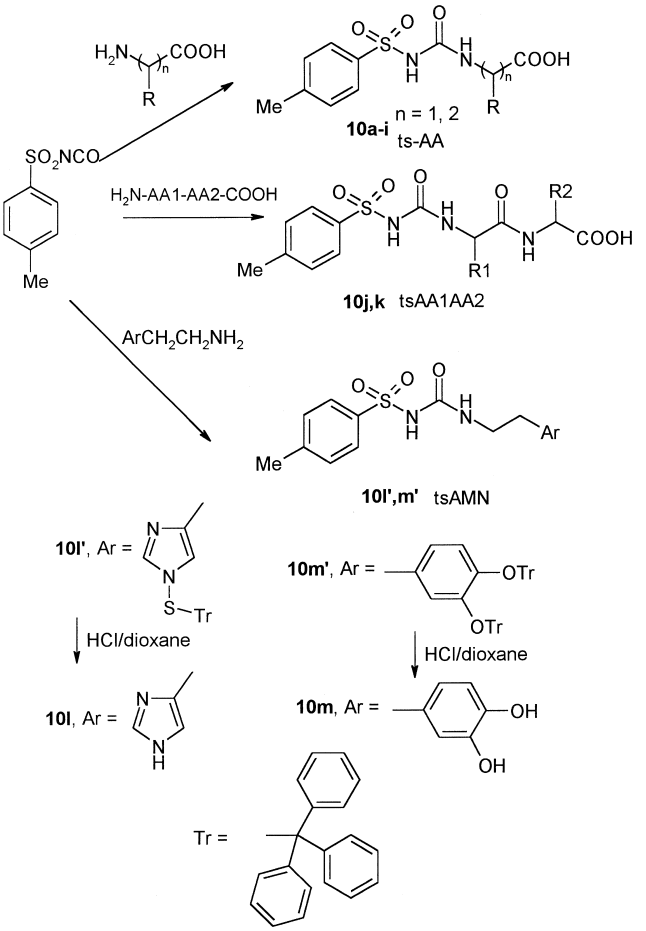
$$PG = 100 \times (\text{Mean OD}_{test} - \text{Mean OD}_0)/(\text{Mean OD}_{ctrl} - \text{Mean OD}_0), \text{ when } (\text{Mean OD}_{test} - \text{Mean OD}_0) \geq 0 \quad (1)$$

$$PG = 100 \times (\text{Mean OD}_{test} - \text{Mean OD}_0)/\text{Mean OD}_0, \text{ when } (\text{Mean OD}_{test} - \text{Mean OD}_0) < 0 \quad (2)$$

where Mean OD_0 is the average optical density measurement of sulforhodamine B (SRB)-derived color just before exposure of cells to the test compound, Mean OD_{test} is the average optical density measurement of SRB-derived color after 48 h exposure of cells to the test compound, and Mean OD_{ctrl} is the average optical density measurement of SRB-derived color after 48 h with no exposure of cells to the test compound. GI_{50} represents the molarity of the compound producing a 50% inhibition of growth of the tumor cells after 48 h exposure to increasing concentrations (10^{-4}–10^{-8} M) of the test compound, measured as outlined before, and this parameter was obtained by interpolation. GI_{50} is in fact the molarity of the inhibitor at which PG = 50%. The standard suforhodamine B (SRB) protein assay was used to estimate cell viability or growth (Teicher, 1997).

3. Results

Reaction of 4-toluenesulfonyl isocyanate with (protected) amines/amino acids/dipeptides afforded the new compounds **10a**–**m** (Scheme 1, Table 1) by the method previously reported by our group for the preparation of carbonic anhydrase inhibitors or activators containing arylsulfonylureido moieties (Scozzafava and Supuran, 1999, 2000b). All these compounds were characterized by spectral and elemental analysis data which confirmed their structure.



Scheme 1.

Table 1
4-Toluenesulfonylureido derivatives **10** prepared in the present study, with their synthetic methods

ts-AA **10a–i** ts-AA1-AA2 **10j,k** ts-AMN **10l,m**

No.	Compound[a]	Synthetic method	M.p. (°C)	Yield (%)
10a	ts-Gly	A	282–3	90
10b	ts-β-Ala	A	250–1	95
10c	ts-Val	A	194–6	94
10d[b]	ts-Lys	A	254–5	83
10e[b]	ts-Arg	A	266–8	90
10f	ts-Phe	A	206–7	86
10g	ts-Tyr	B	221–3	59
10h	ts-DOPA	B	249–50	61
10i	ts-Trp	A	194–6	92
10j	ts-GlyGly	A	235–7	58
10k	ts-β-Ala–His	A	167–8	79
10l	ts-Hsn	C	158–9	67
10m	ts-Dpn	B	173–4	36

[a] ts, *p*-$MeC_6H_4SO_2NHCO$–; this groups acylates the amino-terminal H_2N moiety. When the configuration is not specified, L-amino acid moieties were employed. The usual polypeptide formalism is used: the amino-terminal residue is written first (and is always protected by the ts moiety), whereas the carboxyterminal residue has a free COOH group. Hsn, histamine; Dpn, dopamine. A, amino acid/dipeptide+TsNCO; B, *O*-tritylated amine/amino acid (at the phenolic OH moiety)+TsNCO, followed by deprotection (HCl-dioxane); C, *N*-1-tritylsulfenylated histamine+TsNCO, followed by deprotection (HCl-dioxane); ts should not be confused with Ts, which is *p*-$MeC_6H_4SO_2$.
[b] As hydrochloride salt.

Table 2
In vitro tumor growth inhibition data with some of the new compounds **10j, 10h** and **10m** synthesized in the present work

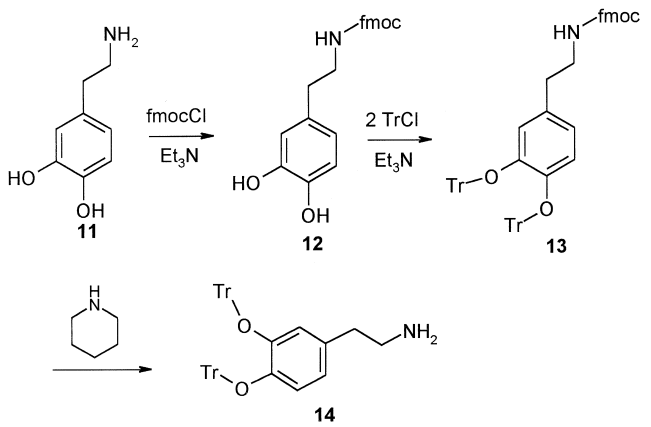
Tumor	Cell line	GI_{50} (μM)[a]		
		10j	**10h**	**10m**
Leukemia	HL-60 (TB)	>100	>100	56
	MOLT-4	>100	>100	58
	K-562	>100	37	>100
	SR	>100	0.1	>100
	CCRF-CEM	>100	25	–
	RPMI-8226	>100	55	38
Non-small cell lung cancer	A549/ATCC	>100	>100	>100
	HOP-62	0.02	>100	>100
	HOP-92	>100	80	>100
Colon cancer	SW-620	>100	24	>100
CNS cancer	SF-268	>100	0.7	>100
Melanoma	LOX IMVI	>100	>100	56
	K-MEL-5	>100	>100	37
Ovarian cancer	OVCAR-8	>100	80	97
Renal cancer	768-0	>100	20	>100
	RXF 393	>100	14	>100
	UO-31	>100	90	>100
Breast cancer	MCF7	>100	>100	69
	MDA-MB-435	>100	>100	50

[a] Molarity of compound producing a 50% inhibition of growth of tumor cells after 48 h exposure to variable concentrations (10^{-4}–10^{-8} M) of the test compound. Errors were in the range ±5–10% (from two determinations).

Tumor cell growth inhibition measurements with compounds **10** were performed by the NIH National Cancer Institute, Bethesda, MD, USA, where the compounds synthesized by us were sent. The following cancer cell types were included in these assays: leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer and breast cancer. The data obtained for the active derivatives are shown in Table 2.

4. Discussion

The reactions of arylsulfonyl isocyanates with active hydrogen containing compounds, such as amines or alcohols/phenols, have been thoroughly investigated due to the many applications of the obtained derivatives as polymers (plastics), insecticides or biologically active substances with potential clinical use (Ulrich, 1965). Recently, we applied this reaction for the preparation of novel types of enzyme inhibitors and enzyme activators (Scozzafava and Supuran, 1999, 2000b,c). 4-Toluenesulfonyl isocyanates react smoothly with amines, amino acids or dipeptides, leading to the corresponding 4-tosylureas with good yields (Ulrich, 1965; Scozzafava and Supuran, 1999, 2000b,c) (Scheme 1). Complications may arise when the nucleophile used in the synthesis contains other reactive groups (such as hydroxy, guanidino, etc.) that might be derivatized by this very reactive isocyanate. Remarkably, the reaction of L-Lys monohydrochloride or L-Arg monohydrochloride with 4-methylphenylsulfonyl isocyanate under the conditions mentioned above led to the formation of only one very pure product, i.e. the α-derivatized compound, without derivatization of the ε-amino moiety in the case of Lys, or the guanidino moiety in the case of Arg, similarly to the case of the related 4-chlorophenylsulfonylureido amino acids reported previously (Scozzafava and Supuran, 2000b). This is probably due to the fact that H^+ acts in this case as a very good side chain protecting group for these two amino acids (Scozzafava and Supuran, 2000b). On the other hand, reaction of tosyl isocyanate with Tyr, DOPA or dopamine led to complex reaction mixtures, in which both the amino as well as the phenolic OH moieties were modified. In order to obtain the pure compounds **10g**, **10h** and **10m**, it was thus necessary to protect the phenolic OH moieties of these nucleophiles. The strategy used is shown in Scheme 2 for the dopamine derivative **14**, but similar approaches have also been employed for the two amino acids (Tyr and DOPA). This consisted of initially protecting the amino



Scheme 2.

group by the fluorenemethyleneoxycarbonyl (fmoc) moiety, followed by tritylation of the phenolic hydroxyls. Selective deprotection of the amino moiety could easily be achieved (without hydrolysis of the *O*-trityl ether bonds) in the presence of morpholine at ambient temperature, thus leading to the *O*-protected derivatives of Tyr, DOPA and dopamine, respectively, which were then used in the reaction with tosyl isocyanate (Scheme 1). The tosylureido derivatives thus obtained were then de-tritylated following the standard procedure, i.e. acidic hydrolysis in dioxane-HCl (conditions in which the tosylureido moiety was not affected).

All tosylureido derivatives **10** prepared in the present study were assayed for inhibition of tumor growth at the NIH National Cancer Institute, Bethesda, MD, USA. Three of these compounds, **10h**, **10j** and **10m**, showed inhibitory properties in vitro against several tumor cell lines (Table 2).

The following should be noted regarding the tumor cell growth inhibition data of Table 2 with the active compounds **10**: (i) different cancer cell lines of the same tumor type possessed a very variable response to inhibition of growth in the presence of the new derivatives. For example, SR leukemia cells were very susceptible to inhibition by **10h** (GI_{50} of 0.1 μM), whereas other leukemia cell lines (such as MOLT-4, HL-60TB) were not inhibited even at concentrations as high as 100 μM of inhibitor. Other leukemia cell lines (such as K-562, CCRF-CEM or RPMI-8226) exhibited intermediate behavior between the two extremes mentioned above, showing 50% inhibition at concentrations of 25–55 μM of test compound. The same situation was observed in the case of diverse non-small cell lung cancer cell lines, with **10j** acting as a very potent inhibitor ($GI_{50}=20$ nM) against the HOP-62 line, whereas the related HOP-92 or A549/ATCC lines showed no measurable levels of inhibition at concentrations of 100 μM of test compound. (ii) All the investigated cancer lines showed some degree of growth inhibition by one or other of the three compounds investigated, but leukemia and CNS cancers seemed to be more prone to inhibition than the other tumors investigated. (iii) Important differences of activity between the three compounds **10** were detected, with the glycylglycine derivative (**10j**) being ineffective in inhibiting a large majority of the investigated tumors, except for the HOP-62 line, for which it showed excellent activity. Broader activity was exhibited by the DOPA (**10h**) and dopamine (**10m**) derivatives, which were effective against a larger range of tumors, with GI_{50} values in the range 0.1–80 μM. Actually, the most effective tumor cell growth inhibitor was the DOPA derivative; its decarboxylation to the dopamine derivative generally produced a loss of antitumor activity. We do not know whether the antitumor activity of these two compounds is associated with their redox properties (oxidation of such derivatives would lead to quinones), but, as suggested by one of the reviewers of this paper, this may be the case since the structurally related tyrosine derivative (**10g**) was completely devoid of antitumor properties. (iv) The inhibition of growth of tumor cells was dependent on the concentration of test compound used in the experiments (data not shown), with growth inhibition increasing at increasing concentrations of tosylureido derivative **10**.

The mechanism of tumor growth inhibition of these compounds is not known at the moment, but presumably they may act as uncouplers of mitochondria, similarly to the diarylsulfonylureas with which they show some structural similarity. Work is in progress in our laboratory to elucidate in detail the mechanism of antitumor action with this new class of tumor cell growth inhibitors, as well as for the design of more effective compounds starting with these three derivatives as lead molecules.

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